Synthesis and DNA binding properties of alkyl-linked bis(benzimidazole) compounds

Yukio Kubota, Hiroki Fujii, Jie Liu and Seiji Tani
Department of Chemistry, Faculty of Science, Yamaguchi University, Yamaguchi 753-8512, Japan

ABSTRACT
We have synthesized novel alkyl-linked bis(benzimidazole) compounds and studied their DNA binding properties by spectroscopic (absorption, CD, flow dichroism and fluorescence) and viscosity measurements. The results indicate that bis(benzimidazole) compounds interact with DNA both by intercalation and by groove binding.

INTRODUCTION
Pentamidine (Fig. 1) was first synthesized in the late 1930s and has been clinically used for AIDS patients with Pneumocystis carinii pneumonia. Tidwell et al. have synthesized a series of dicationic aromatic compounds related to pentamidine to develop more effective antimicrobial agents and have found that 1,4-bis[5-(2-imidazolinyl)-2-benzimidazolyl]butane is more potent and less toxic than pentamidine. Crystallographic and NMR studies of the d(CGCGAATTCGCG)-pentamidine complex have shown that pentamidine is bound in the 5'-AATT minor groove region of the DNA duplex. However, the binding mode of the bis(benzimidazole) to DNA is still not elucidated. To obtain basic information for the development of new antimicrobial drugs, we have synthesized novel alkyl-linked bis(benzimidazole) compounds (Fig. 1) and studied their DNA binding properties by spectroscopic and viscosity measurements.

EXPERIMENTAL
Alkyl-linked bis(benzimidazole) compounds 1–3 (Fig. 1) were synthesized according to the methods described in the literatures and purified by repeated recrystallizations. Calf thymus DNA (DNA; GC=42%) was obtained from Sigma. Spectroscopic (absorption, CD, flow dichroism, fluorescence and NMR) and viscosity measurements were made as previously reported, as a function of the molar ratio of DNA phosphate to drug (P/D). All the measurements were carried out in 5 mM phosphate buffer (pH 6.9, 25°C).

RESULTS AND DISCUSSION
Figure 2 shows the absorption and CD spectra of 2 complexed with DNA. The interaction of 1–3 with DNA results in hypochromism and red-shift in the absorption spectra. No clear isosbestic points were observed (Fig. 2), suggesting the existence of at least two bound species. All the induced CD spectra at high P/D values were very similar in shape to the corresponding absorption spectra. The intensities of the induced CD of 1–3, upon binding to DNA, were weak (Δε=8–40 M⁻¹ cm⁻¹) compared to that of the minor-groove binder Hoechst 33258 (Δε=70 M⁻¹ cm⁻¹), but stronger than those of 2-phenyl-
benzimidazole compounds ($\Delta e < 4 \text{ M}^{-1} \text{ cm}^{-1}$) which bind to DNA by intercalation.$^8$

In order to obtain more details on the binding interaction, we next measured the flow dichroism, viscosity and fluorescence energy transfer. The flow dichroism of all DNA-drug complexes was negative in the absorption region of DNA base pairs (220~300 nm), while almost zero for the drug absorption band (300~420 nm) (Fig. 3). The apparent zero dichroism results from the contribution of two binding modes (intercalation:groove binding=1:2).$^6$ The intrinsic viscosity of sonicated DNA in the presence of drug increased with an increase in the binding ratio. The magnitude of the increase was about one third of that expected for the full intercalation, suggesting the existence of groove-binding mode which does not contribute to the increase of viscosity. The enhancements of relative fluorescence yields due to energy transfer from DNA bases to intercalated drug were observed for all DNA-drug systems.

The results obtained here indicate that the compounds 1~3 interact with DNA by intercalation and groove binding. It is concluded that one of benzimidazole rings of 1~3 may intercalate between DNA base-pairs, but the other or both of benzimidazole rings interact with DNA by groove binding, owing to the steric constraints of alkyl chains.

The solution structures of the DNA complexes with 1 and 2 will be presented in this symposium series.

REFERENCES

6. The compounds 1~3 gave analytical data (C, H, N) in agreement with calculated values and gave spectroscopic data (1H NMR, FTIR and MS) consistent with the assigned structures.